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L11: Entry 4 of 4 File: USPT Jul 30, 1985

DOCUMENT-IDENTIFIER: US 4532215 A
TITLE: Isolation of hepatitis A virus strain HM-175

US Patent No. (1):
4532215

Detailed Description Text (7):
(b) Serial passage. AGMK cells were initially inoculated with 0.1 ml of a 1:20 dilution of stool containing HM-175 virus. For serial passage, the cells were harvested by treatment with trypsin-EDTA (ethylenediamintetraacetic acid), pelleted by low speed centrifugation and resuspended in 4 ml of maintenance medium.

Detailed Description Text (11):
Cells inoculated with the 3-week or 8-week cell harvest of the first AGMK passage (HM-175 M-6, AGMK-1) were positive by IF for viral antigen by 2 weeks after inoculation (Table 2). The cells inoculated with the 3-week harvest remained positive by IF for at least 6 weeks, at which time the cells were harvested with trypsin-EDTA and used for a third cell passage. Whole cells were used because the viral antigen appeared to be cell associated, suggesting that cell-to-cell contact might facilitate infection of the cells.

Detailed Description Text (13):
In the case of the cells inoculated with the AGMK-1, 8-week harvest, granular cytoplasmic fluorescence was observed by one week after inoculation, although only approximately 1% of the cells were positively stained at that time. By 4 weeks, the number of positive cells had increased substantially to 80-90%. Cells were harvested at 6 weeks by treatment with trypsin-EDTA and used for a third passage in cell culture. As with the 3-week harvest above, whole cells were used. Radioimmunoassay (RIA) of this cell harvest yielded a P/N of 8.4. Weak but positive fluorescence was observed in the pass 3 cultures up to time of harvest at 6 weeks after inoculation. Pass 4 cultures were positive at 3 weeks. By pass 5, fluorescence was positive at 1 week and most cells were stained at 2 weeks. Pass 6 cells were positive at 1 week. By 3 weeks, about 60% of the cells were stained. In 7th passage, greater than 90% of the cells were positive by 5 weeks.

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L9: Entry 5 of 6 File: USPT Dec 7, 1993

DOCUMENT-IDENTIFIER: US 5268292 A
TITLE: Reproducible generation of high yields of hepatitis A virus by cell culture

Abstract Text (1):
The invention is a process for cell propagation of hepatitis A virus. The process involves purifying a culture of hepatitis A virus particles. These hepatitis A virus particles are used for infecting cells being cultivated in a media. The hepatitis A particles suitable for use in this invention must have a cytopathic effect in the infected host cells that are selected for culturing the hepatitis A virus. After developing the cytopathic effect in the infected cells, the hepatitis A viruses produce degeneration and lysis in the infected cells and are released into the media. Isolation of the released hepatitis A virus then occurs. The preferred cells for use in this invention are FRhK4 (foetal rhesus monkey kidney) cells. The preferred virus particles for use in this invention are hepatitis A, strain HAS-15 particles have an antigen coded by a nucleotide sequence according to FIG. 1.

Brief Summary Text (7):
A human fecal sample obtained during the acute phase of hepatitis A infection and propagated in FRhK4 cells was designated HAS-15 (Bradley et al., 1984). The quantity of virus produced, as evaluated by radioimmunoassay, appeared to increase with passage, while the time required for viral growth decreased. Subsequently, the ninth passage of this strain was passaged more than 20 times at 7-day intervals to select for a rapidly growing virus population (Wheeler et al., 1986a). Using large scale virus propagation methods, the purified virus derived from these persistently-infected cells was used to characterize the HAV capsid polypeptides (Wheeler et al., 1986b). The initial yield from 350 liters of cell supernatant and the cell lysates from 1.times.10.sup.6 cm.sup.2 of cells was approximately 5 mg of purified virus (Wheeler et al., 1986b), although subsequent recoveries were substantially lower.

Brief Summary Text (11):
There are now four examples of cytopathic infection with HAV isolates which have been reported (Venutti et al., 1985; Shen et al., 1986; Anderson et al., 1986, 1987; and Cromeans et al., 1987), two of which are derived from separate lines of persistently-grown HM-175. It has been suggested that, in the case of one of the HM-175 cytopathic variants (Anderson et al., 1987), that the cytopathic nature and mechanism of persistence is associated with an alteration in the capsid polypeptide composition. Although this possibility cannot be ruled out, nucleotide sequence analysis of the long-term persistently grown HAS-15, which causes cell degeneration when used for an acute infection at high multiplicity of infection revealed that within the first 700 nucleotides of the VP1 molecule or the most surface exposed and potentially variable capsid polypeptide within other picornaviruses, there was only a single nucleotide difference when compared to the nucleotide sequence of lower passage (20 times) HAS-15 (Ovchinnikov et al., 1985).

Brief Summary Text (15):
The preferred cells for use in this invention are FRhK4 (foetal rhesus monkey kidney) cells. The preferred virus particles for use in this invention are hepatitis A, strain HAS-15 particles and which have an antigen coded by a nucleotide sequence according to FIG. 1.

Drawing Description Text (2):
FIG. 1 illustrates a comparison of HAS-15 VP1 nucleotide sequence.

Drawing Description Text (5):
FIG. 4 illustrates a long-term production of HAS-15 HAV in persistently infected FRhK4 cells.

Drawing Description Text (7):
FIGS. 6A-6C illustrate a degenerative effect of a high multiplicity HAS-15 infection in FRhK4 cells at 2 to 3 weeks post-infection.

Detailed Description Text (2):
Approaches to cell culture propagation of hepatitis A virus (HAV) have utilized either acute passage of infected cell lysates/supernatants into uninfected cells or the passage of persistently-infected cells. The data presented demonstrates that the growth and recovery of purified virus from FRhK4 cells persistently infected with HAS-15 HAV decreased over a 2 to 3 month period. In contrast, high multiplicity acute infection of FRhK4 cells with purified HAS-15 HAV resulted in degeneration of the cell monolayer 2 to 3 weeks later. Large scale propagation of acutely infected cells followed by traditional picornavirus purification procedures reproducibly yielded milligram amounts of purified virus. Comparison of the nucleic acid and derived amino acid sequence of VP1 derived from the HAS-15 HAV which caused cell degeneration revealed a single nucleotide difference which results in a homologous amino acid change when compared to published sequences of cloned HAS-15.

Detailed Description Text (3):
The data presented demonstrates that growth of cell culture adapted HAV in an acute, high multiplicity infection reproducibly yields milligram amounts of purified virus. The continued high yield from the use of the acute infection method and the reproducible recovery of purified virus has been facilitated by the fact that HAS-15 is highly adapted to FRhK4 cells (passaged approximately 60 times in persistently infected cells) and that these cells respond to growth stimuli such as the addition of fresh media by increasing the number of cells available for HAV replication and growth.

Detailed Description Text (4):
The process of this invention can be performed by using the materials and methods of the following Example. The following materials and methods represent the elements and procedures of the preferred embodiment of the invention. The Example is better understood by reference to the Figures and their following explanation. FIG. 1 is a comparison of HAS-15 VP1 nucleotide sequence (A) as published by Ovchinnickov et al., 1985, determined from cloned cDNA and (B) generated from viral RNA purified from long term persistently infected cells using primer-directed dideoxynucleotide chain termination. Underlined areas indicate the position of synthetic oligonucleotide primers within the sequence determined, with the fourth primer being located 40 nucleotides further toward the 3' end.

Detailed Description Text (7):
FIG. 4 is a graph of long-term production of HAS-15 HAV in persistently infected FRhK4 cells. Representative samples from FRhK4 cells which were inoculated with cell-culture adapted HAS-15 and carried as persistently-infected cells as described in the results. HAV-Ag present in the media and cells was quantitated by HAV-AG-EIA end point titration. Values are the total amount of HAV-Ag (supernatant and cell lysate) at each of the designated time points.

Detailed Description Text (9):
FIGS. 6A-6C are a photomicrograph of the degenerative effect of high multiplicity HAS-15 infection in FRhK4 cells at 2 to 3 weeks post-infection. FIG. 6A represents FRhK4 cells pass 86; (B) high multiplicity HAV-infected (approximately 300 infectious particles per cell) FRhK4 cells pass 86; and (C) long-term (>2 years) persistently-infected FRhK4 cells.

Detailed Description Text (11):
Large Scale Growth of Persistently Infected Cells. FRhK4 cells, persistently infected with HAS-15, carried by splitting 1:3 every 4 weeks over a three year period, were expanded for large scale virus production. Cell factories with 6000 cm.sup.2 of surface area (Nunc Inc., Denmark) were seeded with these persistently infected cells

from 10 confluent T-150 cm.sup.2 tissue culture flasks (Corning Glassworks, Corning, N.Y.). Cells were released by treatment with 0.05 percent trypsin--0.02 percent EDTA for 15 minutes at 37.degree. C. The trypsinized cell suspension was transferred into 1000 ml of Williams Medium E supplemented with 2 mM L-glutamine, 0.1 mm MEM nonessential amino acids, 0.2 percent sodium bicarbonate, 10 mM HEPES, 50 ug/ml gentamicin, 2 ug/ml of amphotericin B, and 10 percent heat-inactivated fetal calf serum. All media components for cell culture were from Gibco Laboratories, Life Technologies, Grand Island, N.Y. and Chagrin Fall, Ohio, with the exception of fetal calf serum which was from HyClone Laboratories, Logan, Utah. The supplemented medium containing the cells was transferred into a single cell factory and incubated at 37.degree. C. The medium was changed after 2 weeks and cells and medium were then harvested at four weeks. Cells were harvested by trypsinization at room temperature, fresh media supplemented as described above was added to residual cells in the cell factories and the cycle of growth, harvesting, and reseeding repeated.

Detailed Description Text (12):
Large Scale Growth of Acutely Infected Cells. FRhK4 cells (passages 78-84) were grown to confluency (using the media described above) in 850 cm.sup.2 roller bottles (Corning, Corning, N.Y.). Cells were inoculated with the 160S sucrose density fraction of HAS-15 purified from long-term persistently infected FRhK4 cells. The multiplicity of infection was 300 infectious particles per cell as determined by radioimmunofocus assay. The inoculum was diluted in media containing 2 percent fetal calf serum. After rotation at 37.degree. C. for two hours, complete media was added with continued incubation at 37.degree. C. Three days later, acutely infected cells from two 850 cm.sup.2 roller bottles were trypsinized and transferred to 6000 cm.sup.2 Nunc cell factories as described above. The progression of HAV infection was evaluated by microscopic evaluation of a 600 cm.sup.2 single layer Nunc tray containing acutely infected cells. Media was harvested every two weeks, and additional uninfected FRhK4 cells from two 850 cm.sup.2 roller bottles added when cell degeneration was greater than 75 percent.

Detailed Description Text (23):
The purity of HAV (HAS-15) preparations derived from long-term, persistently infected FRhK4 cells was assessed by gel electrophoresis. As shown in FIG. 2A, Lane 2, greater than 98 percent of the silver stained protein is accounted for by the three larger viral polypeptides: VP1, VP3, and VP2. Purified HAV (dilutions of two preparations) was used to evaluate the reproducibility and sensitivity of the HAV-Ag EIA when compared to virus quantitation by the OD.sub.260 method. The data, shown in FIG. 3, indicates that the antibody dependent assay, using purified HAV, is sensitive and reproducible. This HAV-Ag EIA was subsequently used to determine the recovery of virus during purification from cell culture, assuming that 200 picograms of HAV antigen was the lower limit of reliable detection (0.1 OD.sub.490). Evaluation of the number of infectious particles present as determined by the radioimmunofocus assay (RIFA), which measures the synthesis of HAV antigen (HAV-Ag), revealed that approximately one-third of the physical particles were infectious and that the radioimmunofocus assay was 4 to 5 orders of magnitude more sensitive than the HAV-AG EIA for detection of virus.

Detailed Description Text (26):
Under these cell culture conditions, over 99 percent of the virus produced was cell associated while 1 percent was released into the cell culture supernatant (Table 1). Several experiments were performed where persistently infected cells were superinfected with HAS-15. The approach did not result in an increase in virus irrespective of time of culture or concentration of FCS (data not shown). The scale-up of virus production in persistently infected FRhK4 cells from 150cm.sup.2 flasks (data in Table 1) to 6,000 cm.sup.2 factories using 10 percent FCS and a 4 week growth period with refeeding with fresh media after 2 weeks resulted in a 4 to 7 fold increase in HAV recovery when compared to the previously published method for large scale production of virus (Table 2, compare column 1 to columns 2 to 4).

Detailed Description Text (27):
Time Course of Persistent HAV Infection. An evaluation of HAV antigen produced over a 7-month time period by persistently infected cells was performed by inoculating a single T150 flask containing high passage FRhK4 cells (pass 258 at inoculation) with a cell lysate from HAS-15 HAV (pass 23 at inoculation). Two weeks later, the media was harvested and the cells split 1:3. After an additional two weeks growth, the media

from the three flasks was harvested, the cells in one flask split 1:3 while the cells from the remaining two flasks were harvested by scraping, and frozen and thawed three times, sonicated for one minute and the cell debris pelleted by centrifugation at 2000.times.g for 10 minutes. The HAV-Ag present in the harvested cells and supernatant was then evaluated. The results (FIG. 4) indicated that the amount of virus produced during initial infection was substantially higher than during the later period of the persistent infection. Longer term passage of the persistently infected cells resulted in a leveling off of virus production but at much lower levels than in the first three months of infection.

Detailed Description Text (28):
Comparison of Virus Production in Acutely and Persistently Infected Cells. The amount of viral antigen produced in acutely infected FRhK4 cells was compared to that generated in persistently infected cells. Since the data in FIG. 4 suggested that initial infection of cells with HAV resulted in higher levels of virus production, acute infection was performed by inoculating uninfected FRhK4 cells with 300 radioimmunofocus units of purified persistently grown HAS-15 per cell. The amount of virus produced by these two virus-cell systems as assessed by the HAV-Ag EIA are shown in FIGS. 5A and 5B. The results indicated that more virus was produced by the acutely infected cells. The majority of the virus was present in the media while most of the virus within the long-term persistently-infected cells was cell-associated.

Detailed Description Text (29):
In addition to the increased amount of virus produced during the acute infection of FRhK4 cells with virus derived from persistently infected cells, acute infection resulted in degeneration of the cell monolayer approximately two weeks after infection (FIG. 6, Panel B), when compared to uninfected cells (FIG. 6, Panel A). For comparative purposes, the long term high passage persistently infected FRhK4 cells grown under the same conditions are shown in FIG. 6, Panel C. Attempts at superinfection of the high passage FRhK4 cells persistently infected with HAS-15 has consistently failed to induce cell degeneration. In addition, inoculation of BSC-1 cells (passage 80) with 300 infectious particles per cell failed to induce any cell degeneration over a one month period, suggesting that this degeneration effect is virus-cell specific.

Detailed Description Text (30):
Virus Yield from Large Scale Propagation of Acutely Infected Cells. The quantity of HAV produced from FRhK4 cells acutely infected with HAS-15 was 5 to 10 fold greater than the amount of virus obtained from the same number of persistently infected cells (Table 2). In practice, there was little need to lyse cells because of the high degree of cell destruction that occurred over the 4 week culture period and supernatant media alone was used for virus purification. The purity of the recovered virus was assessed by silver staining of PAGE separated polypeptides and revealed the presence of the three viral capsid proteins (FIG. 2B, Lane 1) and no other protein bands that reacted with rabbit anti-160S sera after Western blotting (data not shown).

Detailed Description Text (31):
Nucleotide Sequence Analysis of Long-Term Persistently Grown HAS-15 HAV. A partial nucleotide sequence of HAS-15 HAV from cloned cDNA indicated that the VP1 molecule contained an eighteen nucleotide deletion near the amino terminus (Ovchinnikov et al., 1985). This deletion is unique to this strain when compared to other HAV isolates sequenced thus far (Najarian et al., 1985; Linemeyer et al., 1985; and Baroudy et al., 1985). The virus used by Ovchinnikov et al., (1985) for sequencing was obtained after approximately 20 cell culture passages. The virus obtained from persistently infected cells and used for the acute infection of FRhK4 cells has been passaged approximately 60 times. This same virus also produces cell degeneration in culture. The sequence of the first 700 nucleotides of the VP1 molecule of the passage 60 HAS-15 using primer-directed sequencing of the viral RNA was compared to the sequences obtained by Ovchinnikov et al., (1985) using cloned cDNA (passage 20 HAS-15) and is shown in FIG. 1. The data reveal that the nucleotide sequence is highly conserved, with a single A to G nucleotide substitution in the same region as the eighteen nucleotide deletion. This substitution results in a homologous amino acid change from isoleucine to methionine. The eighteen nucleotide deletion within HAS-15 generates a VP1 molecule which migrates distinguishably faster on polyacrylamide gels compared to other characterized isolates of HAV, i.e., HM-175 and MS-1 (unpublished observation,

Robertson, et al., 1987).

CLAIMS:

1. A process for cell culture propagation of hepatitis A virus comprising the steps of:

a) acutely infecting susceptible cells with strain HAS-15 hepatitis A viral particles at a multiplicity of infection of approximately 300 infectious viral particles per cell, said infection resulting in cell lysis and subsequent release of viral particles; and

(b) isolating and purifying said released viral particles of step a).

L11: Entry 2 of 4 File: USPT Dec 7, 1993

DOCUMENT-IDENTIFIER: US 5268292 A
TITLE: Reproducible generation of high yields of hepatitis A virus by cell culture

Detailed Description Text (5):
FIGS. 2A and 2B are silver stained SDS - PAGE separated HAV polypeptides. Panel 2A - 1 is sucrose gradient purified 160S HAV from long-term persistently infected FRhK4 cells. 2. Molecular weight markers are: 67Kda (bovine serum albumin), 43Kda (ovalbumin), 30Kda (carbonic anhydrase), 20 Kda (trypsin inhibitor), and 14.4 Kda (lactalbumin). Panel B - 1 is sucrose gradient purified 160S HAV from acutely infected FRhK4 cells. 2. Molecular weight markers are as described above.

Detailed Description Text (11):
Large Scale Growth of Persistently Infected Cells. FRhK4 cells, persistently infected with HAS-15, carried by splitting 1:3 every 4 weeks over a three year period, were expanded for large scale virus production. Cell factories with 6000 cm.sup.2 of surface area (Nunc Inc., Denmark) were seeded with these persistently infected cells from 10 confluent T-150 cm.sup.2 tissue culture flasks (Corning Glassworks, Corning, N.Y.). Cells were released by treatment with 0.05 percent trypsin--0.02 percent EDTA for 15 minutes at 37.degree. C. The trypsinized cell suspension was transferred into 1000 ml of Williams Medium E supplemented with 2 mM L-glutamine, 0.1 mm MEM nonessential amino acids, 0.2 percent sodium bicarbonate, 10 mM HEPES, 50 ug/ml gentamicin, 2 ug/ml of amphotericin B, and 10 percent heat-inactivated fetal calf serum. All media components for cell culture were from Gibco Laboratories, Life Technologies, Grand Island, N.Y. and Chagrin Fall, Ohio, with the exception of fetal calf serum which was from HyClone Laboratories, Logan, Utah. The supplemented medium containing the cells was transferred into a single cell factory and incubated at 37.degree. C. The medium was changed after 2 weeks and cells and medium were then harvested at four weeks. Cells were harvested by trypsinization at room temperature, fresh media supplemented as described above was added to residual cells in the cell factories and the cycle of growth, harvesting, and reseeding repeated.

Detailed Description Text (15):
The 1 percent NP-40 soluble fraction from cells or media was treated with DNase I (10 ug/ml final concentration, Sigma, St. Louis, Mo.) in the presence of 20 mM MgCl.sub.2 for 30 minutes at room temperature. The solution was then digested with trypsin (1 mg/ml final concentration,, Sigma) at 37.degree. C. for 1 hour. After cooling to room temperature, SDS or SLS was added to a final concentration of 1 percent, and the virus pelleted by centrifugation at 30,000 RPM in a Type 35 rotor (Beckman) for 3.5 hours at 11.degree. C. The pellet was resuspended in 50 mM Tris, pH 7.5, containing 100 mM NaCl and 0.002 percent phenol red. Viral aggregates were disrupted by the addition of 200 ul of 1 M dibasic sodium phosphate for each ml of viral solution, followed by neutralization with 100 ul of 1M acetic acid for each 200 ul of dibasic phosphate added. Sodium lauryl sarcosyl was added at a final concentration of 1 percent and the solution was centrifuged at 25,000 RPM in an SW 27 rotor (Beckman) at 15.degree. C. for 8 hours through a 30 percent w/v sucrose cushion prepared in 25 mM Tris acetate, pH 7.5, containing 1M NaCl and 1 percent bovine serum albumin. The material which pelleted through the 30 percent sucrose cushion was resuspended in 50 mM Tris, pH 7.5, containing 100 mM NaCl (TNbuffer) and 1 percent sodium laryl sarcosyl and overlayed onto a preformed linear CsCl gradient (1.079 gm/cc.sup.3 -1.582 gm/cc.sup.3) and centrifuged at 25,000 RPM in an SW 27 rotor at 4.degree. C. for 24 hours. Fractions (1.0 ml) were collected from the top and those fractions positive by enzyme immunoassay for HAV antigen (Wheeler et al., 1986a) and having a buoyant density of

1.30 gm/cc.sup.3 -1.35 gm/cc.sup.3 were pooled, concentrated and dialyzed into TN buffer using a Centriflo cone having a 25,000 MW cut off (Amicon Corp.). The concentrated pool was layered onto a 7.5-45 percent sucrose gradient in TN buffer and centrifuged in an SW41 rotor at 36,000 RPM for 90 minutes at 4.degree. C. Fractions (0.5 ml) were collected from the top of the gradient and those positive for HAV antigen in the 160S region of the gradient were pooled, concentrated, and dialzyed against TN buffer using a Centriflo cone as described above.

US Reference Patent Number (3):
4532215

US Reference Group (3):
4532215 19850700 Daemer et al. 435/237

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L7: Entry 1 of 1 File: USPT Aug 31, 1993

DOCUMENT-IDENTIFIER: US 5241053 A
TITLE: Fused proteins comprising glycoprotein gD of HSV-1 and LTB

Abstract Text (1):
Disclosed are (1) a fused protein comprising heat-labile enterotoxin B subunit and a protein heterologous to heat-labile enterotoxin, (2) a recombinant DNA containing a nucleotide sequence coding for the above fused protein, (3) a transformant harboring the above recombinant DNA, (4) a method for producing the fused protein which comprises cultivating the above transformant, producing and accumulating the above fused protein in a culture, and collecting the fused protein, and (5) a method for purifying a fused protein comprising a herpes simplex virus surface antigen and heat-labile enterotoxin B subunit, which comprises cultivating a transformant harboring a recombinant DNA containing a nucleotide sequence coding for the fused protein, producing an accumulating the fused protein in a culture, collecting the fused protein and subjecting the collected fused protein to purification processes comprising cationic exchange chromatography and gel permeation chromatography.

US Patent No. (1):
5241053

Detailed Description Text (29):
For the separation of fused proteins having both the HSV surface antigenicity and the LTB activity, a combination of ion-exchange chromatography and gel permeation chromatography. As fillers for ion exchange chromatography, cationic exchange resins using a sulfo-group as an exchanging group such as ST-Toyopearl 650 M (Toyo Soda, Japan) are preferable. Sovents for equilibriation of columns include 20 mM phosphate buffer (pH 5.8), and eluate solvents include 20 mM phosphate buffer containing 0 to 1M NaCl (pH 5.8). As fillers for gel permeation chromatography, porous particles such as Sephacryl S-300HR (TM) which may isolate a protein of about 1.times.10.sup.4 to 5.times.10.sup.6 molecular weight, are preferable. As solvents for gel permeation chromatography, 5 to 20 mM phosphate buffer containing 0.5 to 1.5 % NaCl (pH 6.8 to 7.2) is preferable. The fused protein can be purified as described above so that it is substantially free of contaminants. This fused protein can then be administered therapeutically.

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L9: Entry 1 of 1 File: USPT Aug 31, 1993

DOCUMENT-IDENTIFIER: US 5241053 A
TITLE: Fused proteins comprising glycoprotein gD of HSV-1 and LTB

Abstract Text (1):
Disclosed are (1) a fused protein comprising heat-labile enterotoxin B subunit and a protein heterologous to heat-labile enterotoxin, (2) a recombinant DNA containing a nucleotide sequence coding for the above fused protein, (3) a transformant harboring the above recombinant DNA, (4) a method for producing the fused protein which comprises cultivating the above transformant, producing and accumulating the above fused protein in a culture, and collecting the fused protein, and (5) a method for purifying a fused protein comprising a herpes simplex virus surface antigen and heat-labile enterotoxin B subunit, which comprises cultivating a transformant harboring a recombinant DNA containing a nucleotide sequence coding for the fused protein, producing an accumulating the fused protein in a culture, collecting the fused protein and subjecting the collected fused protein to purification processes comprising cationic exchange chromatography and gel permeation chromatography.

US Patent No. (1):
5241053

Detailed Description Text (29):
For the separation of fused proteins having both the HSV surface antigenicity and the LTB activity, a combination of ion-exchange chromatography and gel permeation chromatography. As fillers for ion exchange chromatography, cationic exchange resins using a sulfo-group as an exchanging group such as ST-Toyopearl 650 M (Toyo Soda, Japan) are preferable. Sovents for equilibriation of columns include 20 mM phosphate buffer (pH 5.8), and eluate solvents include 20 mM phosphate buffer containing 0 to 1M NaCl (pH 5.8). As fillers for gel permeation chromatography, porous particles such as Sephacryl S-300HR (TM) which may isolate a protein of about 1.times.10.sup.4 to 5.times.10.sup.6 molecular weight, are preferable. As solvents for gel permeation chromatography, 5 to 20 mM phosphate buffer containing 0.5 to 1.5 % NaCl (pH 6.8 to 7.2) is preferable. The fused protein can be purified as described above so that it is substantially free of contaminants. This fused protein can then be administered therapeutically.